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STATE-OF-THE-ART PAPER

Rebuilding the Damaged Heart

The Potential of Cytokines and Growth Factors in the Treatment of Ischemic Heart Disease

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Cytokine therapy promises to provide a noninvasive treatment option for ischemic heart disease. Cytokines are thought to influence angiogenesis directly via effects on endothelial cells or indirectly through progenitor cell-based mechanisms or by activating the expression of other angiogenic agents. Several cytokines mobilize progenitor cells from the bone marrow or are involved in the homing of mobilized cells to ischemic tissue. The recruited cells contribute to myocardial regeneration both as a structural component of the regenerating tissue and by secreting angiogenic or antiapoptotic factors, including cytokines. To date, randomized, controlled clinical trials have not reproduced the efficacy observed in pre-clinical and small-scale clinical investigations. Nevertheless, the list of promising cytokines continues to grow, and combinations of cytokines, with or without concurrent progenitor cell therapy, warrant further investigation. (J Am Coll Cardiol 2010;56:1287–97) © 2010 by the American College of Cardiology Foundation

Cytokine therapy is a promising, noninvasive treatment approach that may prevent cardiomyocyte loss or regenerate damaged tissue. It may also be useful as an adjunctive treatment to mechanical revascularization or cell therapy and for patients with disabling ischemia despite optimal medical treatment. Cytokines are thought to benefit the damaged heart through direct effects in the myocardium and indirectly by stimulating progenitor cells. Progenitor and stem cells reside in the myocardium or are mobilized from the bone marrow in response to cardiac ischemia, and numerous reports indicate that these cells can be mobilized therapeutically by cytokines (1–14). In the ischemic heart, progenitor cells can form a structural component of the regenerating tissue—e.g., resident side-population cells (15) and lineage-negative, c-kit–positive cells (4) differentiated into endothelial cells, smooth-muscle cells, and cardiomyocytes in a murine model of myocardial infarction (MI). The recruited cells also secrete angiogenic or anti-apoptotic factors (16,17) that can maintain myocardial viability, accelerate the recovery of ischemic myocardium, or amplify function in nonischemic regions (18,19). These observations have led to the development of strategies that mobilize progenitor cells and enhance progenitor cell recruitment, thereby preserving or replacing injured tissue (4). Importantly, cytokines are pleiotropic, and their effects can be either beneficial or detrimental (Table 1) depending on the dose and timing of administration. This review focuses on several cytokines that have displayed potentially beneficial effects in pre-clinical or clinical studies of ischemic heart disease.

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Cytokine Agents

Fibroblast growth factor (FGF). The FGF family comprises 22 polypeptides that promote angiogenesis and arteriogenesis by modulating the phenotypes of endothelial cells and vascular smooth muscle cells. FGF4 is encoded by the heparin-binding secretory-transforming proto-oncogene and is not expressed in normal adult tissue. However, when administered to ischemic hearts, FGF4 stimulates endothelial cell proliferation and the secretion of metalloproteinases, urokinase-type plasminogen activator, and vascular endothelial growth factor (VEGF), which subsequently stimulate angiogenesis (20).

The AGENT (Angiogenic Gene Therapy) trials (Table 2) examined the effects of FGF in patients with coronary artery disease (CAD) and medically refractory angina. In the first AGENT trial, intracoronary delivery of an adenovirus coding for FGF4 transcription (Ad5FGF-4) was safe and appeared to improve exercise treadmill times (21). The phase 2 AGENT 2 trial tested whether Ad5FGF-4 treatment improved regional myocardial perfusion (22). Patients administered Ad5FGF-4, but not placebo,

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Abbreviations and Acronyms

Ad5FGF = adenoviral fibroblast growth factor
Ang = angiopoietin
CAD = coronary artery disease
CCS = Canadian Cardiovascular Society
CFI = collateral flow index
EPC = endothelial progenitor cell
EPO = erythropoietin
FGF = fibroblast growth factor
GCSF = granulocyte colony-stimulating factor
GH = growth hormone
GMCSF = granulocyte-macrophage colony-stimulating factor
HGF = hepatocyte growth factor
HIF = hypoxia-inducible factor
HSC = hematopoietic stem cell
IGF = insulin-like growth factor
LVEF = left ventricular ejection fraction
MI = myocardial infarction
PCI = percutaneous coronary intervention
PlGF = placental growth factor
rhVEGF = recombinant human vascular endothelial growth factor
SCF = stem cell factor
SDF = stromal cell-derived factor
VEGF = vascular endothelial growth factor

experienced a substantial reduction in reversible and total perfusion defect, but the results were not statistically significant. The phase 3 AGENT 3 and 4 trials (23,24) enrolled patients with Canadian Cardiovascular Society (CCS) class 2 to 4 angina who were unsuitable for mechanical revascularization. Both trials were halted prematurely when a planned interim analysis of the AGENT 3 cohort indicated that the between-group difference in the primary end point (treadmill exercise duration 12 weeks after treatment) would not reach statistical significance. Differences in secondary outcomes (such as change in CCS class or other clinical variables) were also nonsignificant.

Vascular endothelial growth factor (VEGF). The VEGF cytokines were among the first to display protective or regenerative effects in cardiac tissue. During hypoxia, the therapeutic benefit of VEGF occurs primarily through the stimulation of endothelial cell proliferation, migration, and survival, which subsequently leads to neovascularization (25–30). The extent of regeneration is determined by the tissue retention of the administered isoform and by the isoform's affinity for VEGF receptors. Both endothelial cells and hematopoietic stem cells (HSCs) express VEGF receptors 1 and 2 (also known as Flt-1 and Flk-1, respectively) (31,32), and the expression of VEGF receptors on HSCs appears to be critical for VEGF-dependent regulation of endothelial progenitor cells (EPCs) (33,34).

Treatment with VEGF protein-enhanced collateral blood flow in animal models of chronic myocardial ischemia (35–38) and the safety and feasibility of VEGF therapy for angiogenesis has been assessed in several phase 1 studies (39–44). The VIVA (Vascular Endothelial Growth Factor in Ischemia for Vascular Angiogenesis) trial was among the first large, phase 2 trials (Table 3) completed. Patients with myocardial ischemia who were considered unsuitable for mechanical revascularization were randomized to receive placebo, low-dose recombinant human vascular endothelial growth factor (rhVEGF), or high-dose rhVEGF by intracoronary infusion, followed by peripheral infusions 3, 6, and 9 days later (45). VEGF therapy was safe, but changes in exercise treadmill time (the primary end point) did not differ significantly between groups at the 2-month follow-up visit. Four months after treatment, the only statistically significant finding was the proportion of high-dose VEGF patients who improved by at least 1 CCS class ($p = 0.05$ vs. placebo). In the Euroinject One trial (46), patients with CCS class 3 or 4 angina were randomized to receive 0.5-mg injections of either VEGF-A_{165} plasmid or a placebo plasmid into myocardial regions that displayed stress-induced perfusion defects. At the 3-month follow-up visit, the perfusion defects did not differ between treatment groups, but VEGF treatment was associated with improvements in local wall motion. Intracoronary VEGF gene transfer was also evaluated in the KAT (Kuopio Angiogenesis Trial) (47). Upon initiation of percutaneous coronary intervention (PCI), patients with CCS class 2 or 3 angina (90% of whom received stents) were randomized to receive intracoronary injections of adenovirus-encoded $VEGF_{165}$, $VEGF_{165}$ plasmid liposome, or Ringer's lactate. Six months after treatment, there were no significant differences among the 3 treatment groups in functional status, but myocardial perfusion improved in patients treated with adenoviral VEGF.

Granulocyte colony-stimulating factor (GCSF). GCSF is a potent hematopoietic cytokine that influences the development and function of granulocytes and mobilizes progenitor cells from the bone marrow (48). Progenitor cell mobilization appears to be initiated when GCSF binds to receptors on the cell surface, which leads to the release of enzymes that digest adhesion molecules (49). GCSF also directly influences the activity of some nonhematopoietic cells, such as cardiomyocytes and endothelial cells (50). When administered shortly after MI, GCSF activates the Janus kinase/signal transducer and activator of transcription pathway, which stimulates the production of several anti-apoptotic proteins, decreases cardiomyocyte death, and limits infarct size (51). In a murine model of MI, GCSF treatment was associated with improvements in left ventricular function and enhanced arteriogenesis (52). However, GCSF can also stimulate the differentiation of lineage-committed progenitor cells into neutrophils and macrophages (53), which could worsen inflammation and cardiac remodeling (54).

In phase 1 trials, GCSF administration after PCI for acute MI appeared to improve cardiac function (50,55–58). Patients in the randomized, open-label FIRSTLINE-AMI (Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction) trial received 6 daily subcutaneous GCSF injections starting within 90 min after primary PCI for ST-segment elevation MI; the control group did not receive placebo injections but had identical post-interventional care. Four months (56) and 1 year (55) after the PCI procedure, improvements in

Table 1 Agents

Agent	Molecular Targets	Effects/Mechanism	Potential Detrimental Effects
VEGF	VEGF receptors on endothelial cells, monocytes, and HSCs	Stimulates proliferation, migration, and tube formation Mobilizes EPCs; improves EPC survival and differentiation	Tumorigenesis Retinopathy Flushing (protein infusion) Hypotension (protein infusion)
PlGF	VEGF receptor 1	Cross-talk with VEGF receptor 2 Mobilizes EPCs and HSCs	Associated with carotid atherosclerotic plaque destabilization and adverse outcomes in ACS Associated with depressed LV function in ischemic cardiomyopathy (exact role undefined)
FGF	FGF receptors on endothelial cells, smooth muscle cells, and myoblasts	Stimulates proliferation	Membranous nephropathy Dose-related hypotension Coronary plaque destabilization Accelerated atherosclerosis
GCSF	GCSF receptors on hematopoietic and nonhematopoietic cells	Inhibits apoptosis Activates the JAK-STAT pathway Synergistic with SDF-1 Mobilizes HSCs and granulocytes Accelerates wound healing post-MI Possible effects on resident cardiac stem cells	Tumorigenesis Dysregulated inflammation leading to impaired wound healing or plaque destabilization Medullary bone pain
GMCSF	GMCSF receptors on granulocyte and monocyte precursor cells and monocytes	Stimulates arteriogenesis Activates monocytic cells Mobilizes EPCs and HSCs	Infarct expansion through alteration of inflammation (i.e., dendritic cell function) Plaque destabilization/acute MI "First-dose reaction": dyspnea, hypotension, hypoxia, tachycardia, or syncope Bone pain Peripheral edema, pericardial effusion
SCF	c-kit	Synergistic with colony-stimulating factors Mobilizes bone marrow precursor cells	Mast cell degranulation/wheal formation at injection site Hyperpigmentation Allergic-like reaction including respiratory distress
Angiopoietin-1	TIE2 receptors on endothelial cells	Enhances vessel maturation and stability Mobilizes EPCs and HPCs	Enhanced renal inflammation and fibrosis Pulmonary hypertension Adverse vascular remodeling or angiogenesis
HGF	c-Met receptor on numerous cells (e.g., endothelial cells, cardiac myocytes, progenitor cells)	Attracts resident cardiac stem cells	Tumorigenesis Retinopathy
GH/IGF-1	IGF receptor on vascular and satellite cells, cardiac stem cells	Enhances skeletal and cardiac muscle regeneration	Retinopathy Diarrhea Hypotension Hypoglycemia
Erythropoietin	Erythropoietin receptor on HSCs, EPCs, endothelial cells, and cardiac myocytes	Promotes cell survival Mobilizes EPCs	May accelerate death in patients with cancer Hypertension, headache, arthralgias, and nausea MI (rare)

ACS = acute coronary syndrome; EPC = endothelial progenitor cell; FGF = fibroblast growth factor; GCSF = granulocyte colony-stimulating factor; GH = growth hormone; GMCSF = granulocyte-macrophage colony-stimulating factor; HGF = hepatocyte growth factor; HPC = hematopoietic progenitor cell; HSC = hematopoietic stem cell; IGF = insulin-like growth factor; JAK = Janus kinase; LV = left ventricular; MI = myocardial infarction; PlGF = placental growth factor; SCF = stem cell factor; SDF = stromal cell-derived factor; STAT = signal transducers and activators of transcription; TIE2 = Tyrosine kinase with Ig-like loops and Epidermal growth factor homology domains-2; VEGF = vascular endothelial growth factor.

left ventricular ejection fraction (LVEF) were significantly greater in GCSF-treated patients than in the control group. Unfortunately, these promising results were not reproduced in subsequent double-blind, placebo-controlled trials (59–61) (Table 4).

Several nonrandomized studies have investigated the use of GCSF for treatment of chronic ischemic heart disease (Table 5). Hill et al. (62) administered 5 daily subcutaneous injections of GCSF to patients with CAD and angina. The treatment produced large increases in the number of circulating progenitor cells, but there was no improvement in LVEF, left ventricular wall motion, myocardial perfusion, or treadmill exercise time 1 month after treatment. Wang et al. (63) administered subcutaneous injections of GCSF for 6 days to 13 prospectively selected patients with severe occlusive CAD. CCS class improved from baseline to the 2-month follow-up visit, but the number of single-photon emission computed tomography image segments with perfusion defects either at rest or under stress was unchanged and LVEFs declined, perhaps because GCSF-mobilized leukocytes increased inflammation and fibrosis. Notably, progenitor cell mobilization was considerably lower in 4 patients who were considered "poor mobilizers" than in the 9 remaining patients who were considered "mobilizers."

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Table 2 Randomized, Double-Blind Trials of FGF

	Grines et al. (21) Agent	Grines et al. (23) Agent 2	Henry et al. (24) Agent 3	Henry et al. (24) Agent 4
Phase	1	2	3	3
Patients	Chronic CAD, LVEF ≥40%, CCS class 2–3	Chronic CAD, LVEF ≥30%, CCS class 4	CAD not requiring immediate revascularization, LVEF ≥30%, CCS class 2–4 (U.S.)	"No-option" CAD patients, LVEF ≥30%, CCS class 2–4 (Europe, Latin America, Canada)
Therapy	Ad5FGF-4	Ad5FGF-4	Ad5FGF-4	Ad5FGF-4
Delivery route	Intracoronary	Intracoronary	Intracoronary	Intracoronary
Duration of follow-up	12 weeks	8 weeks	12 weeks	12 weeks
Primary end point	Safety and feasibility	Δ RPDS	Δ ETT	Δ ETT
Assessment modality	Clinical and exercise treadmill test	Adenosine SPECT	Exercise treadmill test	Exercise treadmill test
Secondary end point	Δ Exercise time	Δ Defect size	Δ CCS class, coronary events, or death at 1 year, Δ QOL, time to ST-segment depression during exercise, proportion of patients with ≥30% increase in ETT	
Treatment groups	FGF (n = 60) Placebo (n = 19)	FGF (n = 35) Placebo (n = 17)	High-dose (n = 140) Low-dose (n = 137) Placebo (n = 139)	High-dose (n = 35) Low-dose (n = 43) Placebo (n = 38)
Dose	$10^{8.5}$–$10^{10.5}$ vp	10^{10} vp	High-dose: 10^{10} vp Low-dose: 10^{9} vp Placebo = NA	High-dose: 10^{10} vp Low-dose: 10^{9} vp Placebo = NA
Findings	Trend toward ↑ in exercise time with therapy	Lower rates of revascularization at 1 year with therapy. ↓ in RPDS with therapy from baseline to follow-up*; no change in CCS score (not primary end point)	Potential therapeutic benefit among older patients with more severe angina Pooled analysis: placebo effect much greater in men than women. Women had improved CCS class and ETT with therapy*	—
Notes	—	One outlier in placebo group had 50% ↓ in RPDS	Enrollment ended early when interim analysis indicated that the primary end point was unlikely to differ significantly between groups	

*$p \leq 0.05$.

Ad = adenoviral; CAD = coronary artery disease; CCS = Canadian Cardiovascular Society; ETT = exercise treadmill time; FGF = fibroblast growth factor; LVEF = left ventricular ejection fraction; NA = not applicable; QOL = quality of life; RPDS = reversible perfusion defect size; SPECT = single-photon emission computed tomography; vp = viral particles; Δ = change; ↑ = increase; ↓ = decline.

The mobilizers, but not the poor mobilizers, experienced significant improvements in nitroglycerin use and angina frequency. These findings indicate that the potential benefit of GCSF therapy requires progenitor cell mobilization.

GCSF treatment did not worsen inflammation in several studies of patients with acute MI (50,55,56,58–61), and the rate of restenosis did not differ significantly between GCSF and control patients in the randomized, open-label, FIRSTLINE-AMI trial (55,56) or in 3 randomized, double-blind, placebo-controlled studies (i.e., the STEMMI [Stem Cells in Myocardial Infarction], REVIVAL-2 [22 Regenerate Vital Myocardium by Vigorous Activation of Bone

Table 3 Phase 2 VEGF Trials

	Henry et al. (45) VIVA	Hedman et al. (47) KAT	Kastrup et al. (46) Euroinject One
Patients	Stable angina, "no-option" CAD	Stable CAD undergoing PCI	Severe, stable angina; "no-option" CAD
Therapy	Recombinant human VEGF	Ad or PL VEGF	Plasmid VEGF
Duration of follow-up	60 days, 120 days	6 months	3 months
Primary end point	Δ ETT at 60 days	Minimal lumen diameter, % stenosis	Δ Myocardial perfusion
Secondary end points	Δ ETT, angina, and myocardial perfusion at day 120	Myocardial perfusion, exercise tolerance, incidence of new cardiac events, revascularization, functional class	Safety, wall motion, LVEF, angina
Treatment groups	High-dose (n = 59) Low-dose (n = 56) Placebo (n = 63)	Adv (n = 37) PL (n = 28) Placebo (n = 38)	$VEGF\text{-}A_{165}$ (n = 40) Placebo (n = 40)
Dosage	High-dose: 50 ng/kg per min IC for 20 min† Low-dose: 17 ng/kg per min IC for 20 min†	Adv: 2×10^{10} PFU IC PL: 2,000 μg IC	$VEGF\text{-}A_{165}$: 0.5 mg
Findings	↓ in angina class at day 120 in high-dose group compared with placebo*; no benefit in ΔETT	↑ in perfusion at 6 months compared with baseline in Ad group*; no differences among groups	Improved regional wall motion in VEGF group compared with placebo*

*$p \leq 0.05$. †Followed by 4-h infusions on days 3, 6, and 9.

Adv = adenovirus vector; IC = intracoronary; PCI = percutaneous coronary intervention; PFU = plaque-forming unit; PL = plasmid liposome; VEGF = vascular endothelial growth factor; other abbreviations as in Table 2.

Table 4 Randomized Controlled GCSF Trials* in Patients With Acute MI

	Valgimigli et al. (58)		Ince et al. (56) FIRSTLINE-AMI		Ripa et al. (60) STEMMI		Zohlnhöfer et al. (61) REVIVAL-2		Engelmann et al. (59) G-CSF-STEMI	
Treatment Group	GCSF	Control	GCSF	Control†	GCSF	Control	GCSF	Control	GCSF	Control
GCSF dosage	5 μg/kg/day for 4 days beginning 37 ± 66 h after symptom onset		10 μg/kg/day for 6 days beginning 85 ± 30 min after reperfusion		10 μg/kg/day for 6 days beginning 1–2 days after AMI		10 μg/kg/day for 5 days beginning 5 days after AMI		10 μg/kg/day for 5 days beginning 6 h to 7 days after symptom onset	
Duration of follow-up	6 months		4 months		6 months		4–6 months		3 months	
n	10	10	25	25	39	39	56	58	23	21
LVEF	Increased	Increased	***Increased***	Unchanged	Increased	Increased	Increased	Increased	Increased	Increased
Perfusion	Increased	Increased	NR	NR	NR	NR	Increased‡	Increased‡	Unchanged	Unchanged
LVEDV	Unchanged	Unchanged	***Unchanged***	Declined	Increased	Increased	Decreased	Decreased	Increased	Increased
LVESV	NR	NR	NR	NR	Decreased	Decreased	Decreased	Decreased	Decreased	Decreased
Wall thickening	NR	NR	***Increased***	Increased	Increased	Increased	NR	NR	Increased	Increased

Parameters displaying significantly better performance than was observed in the alternative treatment group are identified with ***bold italicized*** text. Within-group changes from baseline to follow-up were either insignificant ($p > 0.05$) or the significance was not reported. *Double-blind: Ripa et al. (60), Zohlnhöfer et al. (61), and Engelmann et al. (59); single-blind: Valgimigli et al. (58); open-label: Ince et al. (56). †No placebo treatment. ‡Decreased infarct size.

AMI = acute myocardial infarction; GCSF = granulocyte colony-stimulating factor; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume; NR = not reported.

Marrow Stem Cells], and G-CSF-STEMI [Granulocyte Colony-Stimulating Factor ST-Segment Elevation Myocardial Infarction] trials) (59–61). However, Kang et al. (64) found restenosis in 2 of 3 patients who received GCSF alone as adjunctive therapy to primary PCI and in 5 of 7 patients who received both GCSF and infused progenitor cells as adjunctive treatments. This may have occurred because PCI was performed 4 days after GCSF injection, when GCSF-induced leukocytosis and the subsequent inflammatory response was peaking. In an uncontrolled study (65), patients received GCSF therapy 2 days after primary PCI, followed 4 days later by the collection and intracoronary injection of mobilized progenitor cells; restenosis was reported in 8 of 20 patients, and 2 patients experienced MI between 2 and 6 months after treatment.

Among 122 patients in 4 studies who received GCSF therapy soon after acute MI (50,57,60,61), 2 died during the follow-up periods (50,61), another experienced subacute stent thrombosis (60), and a fourth patient underwent emergency splenectomy for spontaneous splenic rupture (57). Wang et al. (63) found no serious vascular adverse events in 13 patients who received GCSF therapy for treatment of severe ischemic heart disease. However, Hill et al. (62) and Boyle et al. (66) reported serious vascular adverse events in 2 of 16 patients with CAD and in 1 of 5 patients with chronic ischemic heart disease, respectively, after GCSF treatment. There is no evidence of GCSF-induced arrhythmia in clinical trials of patients with ischemic heart disease, and the results from some animal studies suggest that GCSF treatment may improve electrical stability (67).

Granulocyte-macrophage colony-stimulating factor (GMCSF). GMCSF stimulates the growth and differentiation of granulocyte and macrophage precursor cells, induces peripheral monocytosis, impedes monocyte apoptosis (5,68), and increases the number of circulating EPCs, which may then participate in regenerative activity (69). In patients with CAD who received a single intracoronary injection of GMCSF, followed by subcutaneous injections every other day for 2 weeks afterward, GMCSF was associated with

Table 5 GCSF Trials in Patients With Chronic Ischemic Heart Disease

	Wang et al. (63)		Hill et al. (62)	Boyle et al. (66)
Treatment Groups	GCSF	Control	GCSF	GCSF
GCSF dosage	5 μg/kg/day for 6 days		10 μg/kg/day for 5 days	10 μg/kg/day for 4 days
Cell therapy	None		None	Intracoronary infusion
Duration of follow-up	2 months		1 month	12 months
End point assessment	SPECT, MRI, echocardiography		MRI	Angiography
n	13	16	16	5
LVEF	Decreased	Decreased	Decreased	Increased
Perfusion	Unchanged	Unchanged	Increased	*Increased*
LVEDV	Decreased	Increased	NR	NR
LVESV	Increased	Increased	NR	NR
Myocardial ischemia	Decreased	Increased	Increased	*Decreased*

Significant ($p < 0.05$), within-group changes from baseline to follow-up are identified with *italicized* text.

MRI = magnetic resonance imaging; SPECT = single-photon emission computed tomography; other abbreviations as in Table 4.

significant improvement in electrocardiographic signs of myocardial ischemia during coronary balloon occlusion and with significant improvement in collateral flow index (CFI). CFI was unchanged in patients who received placebo injections. These benefits likely evolved from cytokine-induced angiogenesis or changes in collateral vascular tone, rather than through a progenitor cell–mediated mechanism, because the mobilization of progenitor cells was low. Improvements in CFI were also reported in a subsequent study of GMCSF therapy, but 2 of 7 GMCSF-treated patients experienced acute MI during the 2-week course of therapy (70). Furthermore, in animal studies of MI, cardiac remodeling worsened after treatment with romurtide to induce GMCSF expression (68,71). Thus the mechanism of action and potential inflammatory consequences associated with GMCSF must be better understood and controlled before larger human trials can be considered.

Erythropoietin (EPO). EPO is involved in both angiogenesis and progenitor/stem cell development. Numerous tissues produce EPO in response to hypoxia and metabolic stress though a mechanism mediated by hypoxia-inducible factor (HIF)-1, and activation of the EPO receptor inhibits apoptosis (72). EPO is also believed to enhance angiogenesis by increasing the proliferation of endothelial cells and by mobilizing bone marrow–derived cells, including EPCs (73). A long-acting EPO analog, darbepoetin alpha, was safely administered to patients with acute MI but provided no functional benefit (74,75). The administration of EPO to patients with acute MI continues to be investigated in the ongoing HEBE III (Intracoronary Infusion of Autologous Mononuclear Bone Marrow Cells or Peripheral Mononuclear Blood Cells After Primary Percutaneous Coronary Intervention) and REVEAL (Reduction of Infarct Expansion and Ventricular Remodeling With Erythropoietin After Large Myocardial Infarction) trials (NCT00378352, ClinicalTrials.gov) (76).

Growth hormone (GH) and insulin-like growth factor (IGF)-1. GH is synthesized by the anterior pituitary gland, and the binding of GH to receptors in the myocardium increases IGF-1 synthesis (77). Experimental evidence has long suggested a role for the GH/IGF signaling in the myocardium (8). In experimental models of MI, subcutaneous injections of GH significantly increased hypertrophy of the viable myocardium and improved left ventricular systolic function without increasing collagen deposition or fibrosis (78,79). Moreover, cardiac stem cells have been shown to possess growth factor receptors that may participate in the activation of these cells for cardiac repair (9). The effect of GH on myocardial growth, cardiac function, and IGF-1 levels in patients with nonischemic or ischemic cardiomyopathy, and in mixed patient populations, has been investigated in several small studies (80–85). Collectively, the findings suggest that additional investigations with GH or IGF-1 are warranted, despite concerns about retinopathy and other potential long-term side effects.

Angiopoietin (Ang). Ang1, Ang2, and Ang3/4 are believed to control the remodeling and stabilization of vessels during the later stages of adult vascular development (86,87). Ang1 is a Tyrosine kinase with Ig-like loops and Epidermal growth factor homology domains-2 (TIE2) agonist that promotes vessel survival, inhibits vascular damage, suppresses inflammatory gene expression, and stimulates vessel remodeling and angiogenesis (88). In rats, Ang1 administration after acute MI increased vascular density, and the vessels appeared to be relatively mature, with larger-sized lumens (89). In a swine model of chronic ischemia, perfusion improved significantly 4 weeks after Ang1 administration, and the improvement was sustained through week 12 (90). Curiously, Ang1 activity seems to oppose VEGF-induced angiogenesis (91). Ang2 acts as a TIE2 agonist in EPCs, which increases angiogenesis (92), but primarily antagonizes TIE2 in vascular endothelial cells, thereby reducing endothelial integrity, increasing vessel permeability, and inducing vessel destabilization and remodeling through, in part, the suppression of Ang1-mediated activity (93). Thus the influence of Ang2 on endothelial cell activity and, by extension, angiogenesis is complex and context-dependent. To date, none of the angiopoietins have been investigated for treatment of ischemic heart disease in clinical studies.

Hepatocyte growth factor (HGF). HGF is a pluripotent growth factor synthesized by the liver. Both HGF and the HGF receptor c-Met can be found in the heart, and cardiac HGF levels are up-regulated after MI in both animal models and human subjects (94). HGF can induce both pro-angiogenic and antiapoptotic effects and is believed to reduce detrimental remodeling after MI (95). HGF has yet to be investigated for cardiac repair in humans.

Placental growth factor (PlGF). PlGF is a member of the VEGF family of cytokines (96) and directly influences angiogenesis by binding to VEGF receptor-1, transactivating VEGF receptor-2, and enhancing VEGF activity (97). PlGF mediates endothelial cell growth, survival, and migration (98,99); mobilizes hematopoietic progenitor cells from the bone marrow; is chemotactic for monocytes and macrophages (2,100,101); and may induce monocytes to release cytokines that increase the homing of stem cells to the injured myocardium (102). Results from pre-clinical investigations suggest that PlGF can improve myocardial perfusion (11,103); however, adenoviral PlGF therapy was associated with greater atherosclerotic intimal thickening and adventitial neovascularization (104). Before clinical trials can be initiated, additional pre-clinical studies must demonstrate that PlGF can induce neovascularization without worsening atherosclerosis.

Stem cell factor (SCF). SCF is the ligand of c-kit (CD117), a proto-oncogene receptor tyrosine kinase that is expressed on adult HSCs (105–107). Activation of the c-kit receptor (107–110) is required for the mobilization of c-kit–positive HSCs and EPCs, and c-kit–positive cells favorably impact cardiac remodeling both directly, through the

repair and regeneration of infarcted myocardium, and indirectly, through an increase in neoangiogenesis (4,111–117). SCF acts synergistically with colony-stimulating factors to mobilize bone marrow-derived stem cells (106,118), improves the homing of c-kit–positive bone marrow-derived cells (118), and enhances the migration of resident cardiac stem cells to the peri-infarct zone (119). Combined SCF and GCSF therapy increased myocardial blood flow, but not function, after circumflex artery ligation in baboons (120), and treatment with SCF, GCSF, or both (compared with placebo) reduced mortality in a murine model of MI, but SCF therapy alone did not improve left ventricular performance or remodeling (121). The benefits of combined SCF and GCSF treatment have also been reported in a mouse infarct-reperfusion model (122).

Practical Considerations

Timing of therapy. The cellular environment of infarcted myocardium is dynamic, so the timing of cytokine administration is a necessary consideration during treatment. For example, the number of GCSF receptors on cardiomyocytes increases markedly soon after MI in rats (51), and prompt GCSF administration has been associated with declines in cardiomyocyte apoptosis, smaller infarct areas, and decreased ventricular dilation (51,123,124), whereas delayed GSCF treatment aggravated left ventricular remodeling in a porcine myocardial-reperfusion model (124). Four clinical trials of GCSF therapy administered at different time points and for different durations after MI yielded different patterns of efficacy (55,60,61,64).

The time-dependent effects of cytokine administration after MI could be either direct or related to the recruitment of bone marrow-derived progenitor cells. The activity of bone marrow-derived cells may differ depending on the state of the infarcted myocardium, and the expression of cell-signaling molecules may change over time. Wang et al. (30) found that the expression of VEGF and stromal cell-derived factor (SDF)-1, which is involved in the homing of progenitor cells to ischemic tissue, is not acutely elevated in ischemic myocardium (30), and that human plasma levels of SDF-1, VEGF-A, and FGF-2 reach maximum concentrations 2 to 3 weeks after MI (125). In a murine MI model, cytokines involved in progenitor-cell homing, including SDF-1, were up-regulated immediately after MI, then down-regulated over a 7-day period, and the delayed administration of GCSF (56 days after MI) improved remodeling only when SDF-1 expression was enhanced in the infarcted tissue. These observations imply that the effectiveness of cell-mobilizing agents for treatment of MI may depend on the early, cytokine-mediated recruitment of mobilized stem cells to the injured myocardium (126,127).

Safety. Because angiogenic inhibitors can reverse or reduce the formation of atherosclerotic plaques in animal models (128,129), the pro-angiogenic activity of cytokines could, in theory, worsen plaque formation or contribute to plaque destabilization (as noted in the studies of GCSF, GMCSF, and PlGF described above). However, successful angiogenic therapy may enhance re-endothelialization after vascular injury and, consequently, impede plaque formation by suppressing neointimal thickening (130). The long-term theoretical concerns associated with therapeutic angiogenesis include an increased risk for neoplastic disease and the induction or worsening of retinopathy. No evidence of a link between angiogenic cytokines and tumor development or retinopathy has been reported in clinical trials (131), but candidate patients must be adequately screened and monitored for malignancies, premalignant conditions, and retinopathy.

Reperfusion injury. In most published animal studies, MI was induced by ligating the coronary artery, which permanently halts blood flow. However, blood flow is restored in clinical presentations of MI, so the ischemia-reperfusion models used by Beohar et al. (124) and Dawn et al. (122) more closely reproduce the clinical experience. The release of cell-homing factors may differ in chronically ischemic and reperfused cells and could influence the recruitment of progenitor cells to the injured tissue. In addition, early, sustained reperfusion has been shown to reduce left ventricular remodeling after MI (62,132), and this benefit could obscure the effects of cytokine administration in clinical trials.

Summary and Future Directions

Cytokine therapy could provide an attractive treatment option for many cardiovascular diseases. Although the outcomes from clinical trials of cytokine therapy have failed to meet expectations, combinations of cytokines, with or without concurrent progenitor cell therapy, or the administration of agents (e.g., HIF-1 alpha, Sonic hedgehog) that up-regulate several angiogenic factors simultaneously, warrant further investigation. For patients with chronic CAD, therapy that combines progenitor cell-mobilizing agents with the elevated expression of cell-homing factors (e.g., SDF-1) in ischemic tissue may be particularly valuable. The effectiveness of regenerative therapies could also be improved by a more complete understanding of the cellular environment after MI. The growth factors IGF-1 (133) and HGF (134) induce expression of collagen-degrading metalloproteinases that could make the extracellular matrix more amenable to progenitor cell migration, and researchers have begun to investigate other agents that modulate the interstitial matrix, including the matrix protein Del-1, which coordinates integrin expression (135), and Cyr61, which induces angiogenesis by binding to avβ5 (136). Approaches that combine cytokine and cell therapy can be refined by continuing to characterize the relevant signaling pathways, the optimal magnitude of progenitor cell mobilization, the relative efficiency of subpopulations of bone marrow–derived cells, and the potential importance of resident cardiac progenitor cells (9).

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